Open Literature Review

*Note that EFSA has a summary of this study on the recent EFSA published document (see Fryday S, Tiede K and Stein J, 2015. Scientific services to support EFSA systematic reviews: Lot 5 Systematic literature review on the neonicotinoids (namely active substances clothianidin, thiamethoxam and imidacloprid) and the risks to bees. EFSA supporting publication 2015:EN-756, 656 pp. Available online: www.efsa.europa.eu/publications).

PMRA have similar summary of end-point results with EFSA which can be used for the pollinator risk assessments.

1. **Chemical Name:** Thiamethoxam

2. **PC Code:** 060109

3. **CAS No.:** 153719-23-4

4. **MRID:** xxxxxxx

   PMRA: To be determined

5. **ECOTOX Record Number and Citation:** Tremolada P., Mazzoleni M., Saliu F., Colombo M. and Vighi M. 2010. Field trial for evaluating the effects on honeybees of corn sown using Cruiser® and Celest XL® treated seeds. Bull Environ Contam Toxicol 85(3):229-234

   Study Type: Tier III (field study) DACO 9.2.4.7

6. **Purpose of Review:** Thiamethoxam re-evaluation for pollinators/Pollinator risk assessment

7. **Date of Review:** November 17, 2014

8. **Description of Use:** Qualitative (See reviewer's comments below)

9. **Summary of Study Findings:**

Executive Summary
A field study was conducted to investigate the possible adverse effects of corn sown using commercial seeds dressed with thiamethoxam (Cruiser®; 350 g L^{-1}) and Celest xl® (fludioxonil and metalaxyl-M; 25 and 10 g L^{-1}, respectively) on honeybees. Mortality (adult mortality) and behaviour (foraging) were the parameters collected.

Before corn sowing, no significant differences were observed on the number of dead bees in the exposure and control hives ($p = 0.395$). On the day of sowing, bee mortality in the control hives did not significantly differ to that of pre-treatments levels, but bee mortality in the exposure

hives increased from 20 before pre-treatment to >40 on the day of sowing. Shortly after the sowing period, bee mortality in the exposure hives decreased to values of approximately 10. There was a significant effect of treatment ($p=0.024$) and time ($p=0.020$) on mortality.

Foraging bees/minute in both hive groups was similar to previous days on the day of sowing. On subsequent days, the number was reduced in both hive groups but more markedly in treated hives. There was a significant effect of treatment and time ($p<0.001$ for both) on foraging.

Methods

The study was conducted in an agricultural farm situated in the agricultural plain south-east of Milan, Italy. In this farm, a second sowing of corn was performed on a single area of 7 hectares on June 24, 2008 at 11 am. Corn seeds, dressed with Cruiser® and Celest xl®, were sown with a Kinze 3,000 seed drill (pneumatic type). Cruiser contains the insecticide thiamethoxam (350 g L^{-1}) while Celest xl® contains the fungicides fludioxonil (25 g L^{-1}) and metalaxyl-M (10 g L^{-1}) as active ingredients.

Corn was sown using approximately 70,000 seeds $hectare^{-1}$ (21 kg of seeds; 0.3 g $seed^{-1}$). Seeds were dressed at the recommended dose of 100 mL of each product for every 100 kg of seeds (www.syngenta.it), equating to a total mass of active ingredient per hectare of 7.35, 0.525 and 0.21 g for thiamethoxam, fludioxonil and metalaxyl-M, respectively.

Control and exposure hives were deployed within the experimental area at two sites. The control hives were placed inside a farm garden (approximately 200 m away from the treated fields). The exposure hives were located at the field hedge boundary of the test field. Two days prior to sowing, experimental hives were selected. Homogeneous colony consistency was assessed using the Liebefeld method. There were six experimental hives; two exposure hives and four control hives.

Treatments:
Exposure hives = 2 (located at the field hedge boundary of the test field)
Control hives = 4 (inside a farm garden approximately 200 m away from the treated fields)

Homogeneous colony consistency was assessed using the Liebefeld method.

Parameters measured:

*Direct mortality (*direct lethal effects of pesticides inside the hive or in front of it):

Direct mortality in the hive area was monitored using traps ('underbaskets'). Each underbasket has a dimension of 50 x 100 x 10 cm. It comprised of a wooded tray sealed at the base by an iron net to retain falling dead bees and on the top by a second net of a mesh size that allowed dead bees to pass through, but prevented removal of bees via predation. These traps were located on the ground outside the entrance to each hive to collect dead bees that were eliminated from the inside or dead just in front of the hive. Mortality measurements were conducted over a 6 day

period; commenced 2 days prior to seed sowing (June 22) and ended June 28. The number of dead bees in each underbasket was counted and removed at 4:00 p.m. each day.

Foraging activity intensity (sub-lethal effects far from the hive, such as the failure of the foraging activity or loss of orientation):

The foraging activity of honeybees was monitored each day at 11:00 a.m. Assessments were done at the same date when direct mortality was evaluated but with an additional 2 assessment days (July 3 and July 9).

For each hive, sampling was done for 1 min and was repeated three times. Mean daily foraging was calculated. Only foraging bees that entered the hive with pollen were counted during the 1 min sampling period. For each hive, this process was repeated three times, enabling a mean daily foraging value to be calculated.

Statistics
Statistical analyses were performed using SPSS ver. 17.0 software. Statistical analyses comprised of:
- Kolmogorov–Smirnov non-parametric test to verify the normal distribution of the data,
- Student t-test used for single comparisons (ANOVA) for one-factor-multi-comparisons,
- General Linear Model unvaried analysis (GLM) with post-hoc Bonferroni and Duncan tests to assess the interaction between time with respect to the sowing and the hive distance from the treated fields.

Results

Direct mortality (4 days)
Fig. 1a presents the number of dead bees per day for both the exposure and control hives, during the corn sowing period. Before corn sowing, no significant differences were observed on the number of dead bees in the exposure and control hives ($p = 0.395$). On the day of sowing, bee mortality in the control hives did not significantly differ to that of pre-treatments levels, whilst in the exposure hives bee mortality increased from 20 before pre-treatment to >40 on the day of sowing. Shortly after the sowing period, bee mortality in the exposure hives decreased to values of approximately 10. There was a significant effect of treatment ($p=0.024$) and time ($p=0.020$) on mortality.

Time period since sowing and hive group were found to be statistically significant in determining the number of dead bees which indicates that the number of dead bees is largely dependent on the location of the hive (i.e. distance from fields sown with treated corn seeds) and the time period since sowing. The relationship between 'time' and the number of the dead bees was only found to be significant in the exposure hives (ANOVA: $F5;6 = 12.4$; $p = 0.004$).

Foraging activity (16 days)
The mean number of foraging bees carrying pollen and entering in the hive in 1 minute during the corn sowing period is shown in Fig. 1b.

No significant differences was observed on foraging bees entering the hives in the time of 1 min between the two hive groups prior to sowing and on the day of sowing. A day after sowing, the mean number of foraging bees decreased in both hive groups, compared to that of previous days. In the exposure hives, the observed decrease was more evident as the mean number of foraging bees decreased from a mean value of 25 to just 9.3 the day after sowing. In the control hives, the mean number of foraging bees decreased from a mean value of 28 to 23 in the same days. Measurement of foraging activity 15 days after sowing revealed that in control hives, values had recovered to levels similar to that of before sowing. On the contrary in exposure hives, an opposite result was observed: one hive had recovered to 'normal' levels, while the other still presents altered conditions.

Both time and hive group were both statistically highly significant in determining the number of foraging bees entering the hives in 1 min ($p<0.001$ for both).

Control hives were also subjected to adverse effects far from the hives as the number of foraging bees entering in the control hives significantly changed over time ($p = 0.006$).

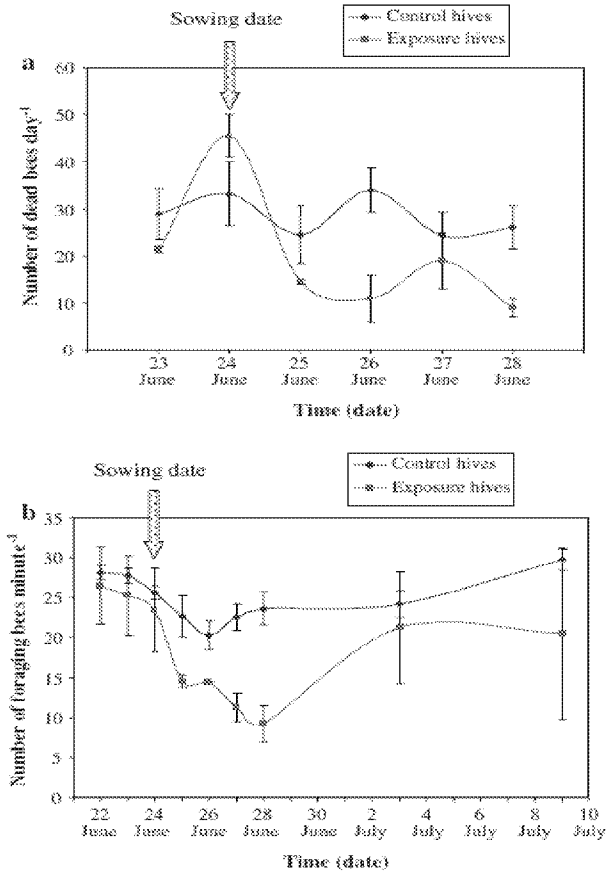

Fig. 1 Mean number of dead bees *per* day during the sowing period in the exposure and control hives (a), and mean number of foraging bees carrying pollen and entering in the hive in 1 min during the sowing period in the exposure and control hives (b). *Bars* refer to the interval of the mean ± standard deviation

Data Quality Evaluation
The data appear to be of good quality.

10. Peer Review

Primary Reviewer Comments

Rationale for Use:

The results from this study show that sowing operations with Cruiser®- and Celest xl®-dressed corn seeds affected exposure hives, while control hives located 200 m away from the test site and protected by a vegetation barrier were much less affected by the pesticide toxicity. After the sowing period in the exposure hives, an increase in the number of dead bees was registered. According to the study author, for an acute toxic effect to be observed, the bees should be in contact with thiamethoxam at a concentration close to or exceeding its LD_{50} value. In contrast, foraging bees can be affected also far from the hives by either lethal or sub-lethal effects.

The authors indicated that the observed effects of this study were too limited to be conclusive for supporting the pesticide cause of colony collapse disorder. The results, however, can be considered as a field proof of the possible toxic effects that seeds dressed with neonicotinoid pose to bees during sowing.

Limitations of Study:

- The duration of the monitoring of honey bee mortality and foraging activity was limited due to weather conditions. The measurements began when the weather was stabilized and were conducted for a period in which the weather was stable, sunny and without evident wind interference. The duration of the study was rather too short.
- No information on the health of the hives.
- Weather conditions were possibly collected but not included.
- Pollen/nectar carried by the bees back to the hives after foraging were not identified.
- Non-inclusion of raw data made it impossible to verify statistical analyses done and to make conclusions.

Description of Use in Document:
Qualitative. The study provides information on the adverse effects of corn seeds dressed with thiamethoxam during sowing to honey bees. The study is considered to be scientifically sound and informative. Results may be considered as one of the lines of evidence in the pollinator risk assessment.

Secondary Reviewer Comments (Barbara Martinovic Barrett, Officer 1183):

- The secondary reviewer agrees with the overall conclusion of the study. The study author recognized that the control hives were likely exposed to the chemicals, but that a greater distance from the sowing appeared to result in lower effects.
- Corn seeds, dressed with Cruiser_ and Celest xl_, were sown with a Kinze 3,000 seed drill (pneumatic type). Cruiser_contains the insecticide thiamethoxam and Celest xl_contains the fungicides fludioxonil and metalaxyl-M as active ingredients. It is unknown if the seed drill is comparable to drilling equipment in Canada.
- The rate for thiamethoxam was 7.35 g ai/ha (based on sowing rate), which is much less compared to Canadian rate for Cruiser 5FS (118.3 g ai/ha for field corn and 75.6 g ai/ha for sweet corn), and thus may represent a conservative scenario.
- Control hive was only 200 m from treated fields. Therefore, there may have been cross foraging, and some contact exposure. The study author suspected that control hives were also exposed to thiamethoxam and fungicides. Residues were not measured to confirm.
- There were two exposure hives and 4 control hives.
- Endpoints included mortality in the hive area and foraging activity intensity. The study focused on acute effects and did not include sublethal or brood parameters.
- Bee mortality measurements were conducted over a 6 day period and started 2 days prior to seed sowing (June 22nd) and finished on June 28th. Therefore, the observation period was short.
- In this study, there was already one sowing of corn made prior to the experiment. It is unknown if bees were already exposed to neonicotinoid treatment prior to the experiment, although it should be noted that pre-experimental mortality was lower compared to post-sowing.
- Except for the day of sowing, the control hives had higher mortality on all other days compared to the treatment hives. However, the trend did appear to show that the "exposure" hives had a greater number of dead bees at the time of sowing, and that foraging was lower following the sowing of corn seeds.

EPA Reviewer: Ryan Mroz, Risk Assessment Process Leader, OPP/EFED/ERB-5 – 1/9/2020

I agree with the qualitative classification by PMRA reviewers. 

Resolution:
None required.

11. References: None

Primary Reviewer (PMRA): Rhomela F. Masangkay, Ph.D. (1312)

Date: November 17, 2014

Secondary Reviewer (PMRA): Barbara Martinovic Barrett, Officer 1183

Date: May 21st 2015